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THE PROBLEM OF DEVELOPMENTAL STAGES OF GROWTH OF
MICROORGANISMS

by N. D. Ierousalimski

In a study of the action of exterior factors on vital functions it is commonly admitted that there exists between them a constant dependance, characterized by three points: minimal, optimal and maximal. Such a simple conception is not always justified. There is nothing astonishing in what there may be in microbiological literature of numerous contradictory contributions on the subject of the influence of conditions of surrounding environment on the process of metabolism, growth and development, and notably on the subject of causes of formation of spores by bacteria.

These problems, as well as certain others which are not clear, are easily resolved in the light of the theory of stadal development studied by soviet biologists. Conforming to this theory all ontogenetic development traverses a series of consecutive stages where the following stage cannot begin until after the completion of the preceding stage. Each stage implies certain specific conditions of medium, without which they cannot go on. In the same way specific conditions are necessary to the formation of each structure or function of the organism. All these conditions determining the progress of ontogenesis are the same to which the species in question has adapted itself. in the process of phylogensis. Apart from these conditions indispensable to its ontogenetic development, the organism is influenced by the action of other factors which modify the time of its growth and development.

By growth is understood the increase of the living mass of the organism, independent of the structural composition by means of which this growth is produced. This is why there is no permanent liaison between the process of growth and that of formation of one or another structure (that is to say the development of the organism). Under the influence of culture medium either the rate or extent of growth can be modified, singly, or the tempo of development alone, or yet again the two together; (the acceleration of growth may be accompanied by a slowing down of development, and vice versa). In order to state the influence of exterior conditions on the organism it is very necessary to take account not only of the rate of the process of growth and development, but also of the relation existing between these processes. This relation is characterized by the degree of development (stage of ontogenesis) attained by the organism for a certain ~~length~~ living mass (independent of time). We call this relation "intensity of maturation" of the organism.

The opinions stated hereabove refer entirely to microorganisms. Let us examine as an example the problem of sponulation in butyric bacteria. Their evolutionary cycle begins with germination of the spore. The structure and physiological functions of the vegetative cell are modified after several divisions. In the midst of its protoplasm is deposited the granulose which turns dark violet with iodine. Among the acids formed, butyric acid begins to predominate over acetic acid, although in young cells this relation was inverse. Also the cell thickens; cylindrical at the start, it narrows finally at the extremities to become fusiform (it takes the form of clostridium). In the clostridium is formed the germ of the future spore; the cell diminishes little by little and undergoes lysis resulting in the formation of a mature spore, round in form. Such is the normal way of development of these bacteria. There exist equally all sorts of pathological modifications and forms in the way of decay which diverge from the normal development.

Table I cites the data which characterize the growth and development of culture of butyric bacteria in three different media: (1) Rich nutritive medium which besides carbohydrates and salts contains an excess of organic nitrogen and all the indispensable vitamins. (2) Potato medium indicator, less rich in nitrogen easily assimilable. (3) Poor nutritive medium whose only source of nitrogen is ammonium phosphate and which contains no traces of vitamins contributed in the course of inoculation.

Table I
Influence of medium on growth and development of butyric bacteria

Medium	Hours				
	2	6	12	20	25
Number of bacterial cells in millions per 1 ml					
Rich	50	184	225	244 cl.	256
Potato	38	100	158 cl.	188	205
Poor	26	42	58	90	112 cl.

Remark: cl. marks the first appearance of the first clostridial forms

As one must expect, the rate of growth and the quantity of the living mass (which is to say the number of cells) correspond to the quantity of nitrogen and vitamins contained in the nutritive media: the growth is most abundant in rich medium, scantiest in the poor medium. This last culture contains an appreciate number of involutive forms: very ~~zix~~ elongated cells, curved inwards, with oxyphile protoplasm. In the rich medium one sees cells with two or three large granules. Cytochemical reactions permit stating that these granules constitute deposits of protein in reserve (not proteides)

We note that in culture the bacterial cells grow and develop with variable rapidity, but the conditions of their life and nutrition are not at all identical. That is why the appearance of clostridial forms and spores as well as the increase in the relative number of these forms (in relation to the total cells in the culture) show the tempo of development of the bacterial culture. If one refers to the moment of the appearance of clostridia (tabl.1) one ascertains that the culture is developed more rapidly on indicator medium and not on rich medium. From all evidence the excess of nitrogenous food in this last medium slows down the development of the culture, although it intensifies its growth. In consequence the intensity of maturation (which is to say the relation between the development and growth) in this medium is reduced. The development of the culture is equally retarded in poor medium because of the acute insufficiency of sustenance. However at the moment of the appearance of the first clostridia the living mass of this culture is inferior to the other two because of its very feeble growth. From this it results that this culture occupies first place for intensity of maturation (by relation between development and growth)

To better specify the intensity of maturation of cultures in different media Figure 1 presents on abscissa the values of the living mass (number of cells) and on the axis of the ordinate the degree (level) of development evaluated according to the relative number of cells in state of sporulation. One must take into account that following the composition of the medium the cultures attain the same level of development for the different living masses. In consequence the intensity of maturation of the cultures varies: it is maximum in a poor medium, and minimum in rich medium.

It is interesting to compare the number of cells actually sporing (that is the forms with the prespores plainly visible and mature spores) with the number of cells which are capable of forming spores. This capacity is manifested by a certain number of morphological and physiological modifications, notably by a granulose deposit in the cell. Table 2 makes evident that the number of cells in rich medium and that on potato exceeds that on poor medium. From all evidence the insufficiency of nitrogenous nourishment and vitamins is not favorable to the acquisition of spore-forming capacity.

Table 2

Influence of medium on sporulation of
butyric bacteria

MEDIUM	Number of cells	
	Capable of forming spores (in percentage relation to total of cells)	actually forming spores
Rich	70	30-40
Potato	70-80	50
Poor	30	20-30

The actual transformation in spores presents an entirely different picture. In poor medium almost the entire lot of cells capable of spore-forming is transformed actually into spores and into clostridia, while in the other two media the sporulation affects only a part of these cells: in potato about two-thirds, on rich medium about half. To all appearances the final stage of sporulation is favored by the lack of nourishment, contrary to conditions favorable to the preparatory stage.

Thus the formation of spores depends on the exhaustion of nutritive reserves, which means that they are found in conditions which are those of the natural habitat of the bacteria. Contradictory data on the influence which medium exerts on sporulation are explained by the following estimation of the fact that the needs of bacteria change with their passage to a new stage of development.

There are certain indications that numerous bacteria are capable of assuming forms infinitely small, invisible to the microscope and passing through sterilizing filters. These filterable forms can, following a certain number of intermediary stages, again become bacterial cells. The question of whether these transformations relate to the process of regeneration or whether to the normal evolutionary cycle of the bacteria has not yet found a solution for lack of deeper study of conditions on which these transformations depend. That is why, before considering these transformations as an evolutionary cycle, it is necessary to find specific conditions of medium, indispensable to the formation of filterable forms as well as to the development of the latter in bacterial cells. The conditions above-mentioned should correspond to those which exist in the natural habitat of the bacteria. It could be that the whole evolutionary cycle of butyric bacteria is itself composed of two successive cycles: 1) spore-vegetative cell - spore, and 2) filterable form - cell - filterable form. The polycyclic development exists in numerous inferior organisms: champignons, algae, protozoa.

On passing through a new stage of development the organism acquires the faculty of forming well-determined structures and of exercising well-defined physiological functions. Most often the conditions of medium indispensable for acquiring the faculties indicated does not correspond to those of effective realization. We have made the same observation for the process of sporulation. As to the physiological functions, the acetone-butylic bacteria furnish an example. The young cells of these bacteria transform the carbohydrates into carbonic acid and hydrogen, with a predominance of the latter, and non-gaseous products, principally acetic acid and butyric acid. The young cells in the period of growth and multiplication progressively form a particular complex of enzymes which we conventionally call "acetonic ability". When this enzymatic system attains a sufficient degree the bacteria obtain the faculty of forming acetone and butanol while transforming carbohydrates and acids. From this fact the concentration of the latter in the medium goes on diminishing since the carbonic acid begins to predominate among the gaseous products. However, this second phase of fermentation cannot actually begin except in certain conditions of medium, different from those in which the bacteria acquired the acetonic capacity. For example in the period of acquiring acetonic capacity, the bacteria need a medium less acid than in the second phase of fermentation (see the first two lines of table 3).

Table 3
Relation of acetone-butyric bacteria to pH of medium

Medium	Age of cultures (in hours)	Value of pH		
		Minimum	Optimum	Maximum
Corn	4	3,8 - 4,0	5,2 - 5,8	6,8 - 7,0
"	18-28	5,2 - 5,4	4,4 - 5,3	6,7 - 6,9
Peptone-glucose	20 - 24	3,9 - 4,0	5,3 - 5,9	7,0 - 7,1

The excess of peptide nitrogen and amine easily assimilable hastens growth, corn slows the development of these bacteria. Thus for example the culture of 20-24 hours on medium of peptone-glucose stays young despite its physiological age. The absence of sporulation and the behaviour with regard to the pH of the medium indicate it. (Table 3)

The more the albumin is decomposed, the more the nutritive medium contains of peptides and amino acids, the more the growth of the culture and the more intense are the production of amines, since the development of the culture is retarded and second phase of fermentation sees its intensity diminish (Table 4)

Table 4
Influence of the degree of proteolysis on fermentation and
growth of acetone-butylic bacteria

Duration of action on albumin of Pepsin — Trypsin —	Maximum quantity of bacterial cells in millions, per ml.	Acidity ml. 0, IN in 1 OM3	Gas in gr.I	Acetone in gr I
0	0	330	3,1	26,2 4,5
16	0	390	3,4	30,2 3,8
120	0	580	5,6	6,0 0,6
120	240	750	6,9	5,2 0,3

The underestimate of stadal development of bacteria is the origin of contradictory beliefs as to the causes of weakening of the second phase of acetone-butylic fermentation.

Acetone can also be produced by acetone-ethyl bacteria. On rich nutritive medium they acquire this ability much more rapidly and easily than butyl-acetone bacteria. But in media with insufficient quantity of Vitamin B the acetonic ability is developed slowly. Vitamin B₁ is the specific agent favoring formation of this ability, since it influences in a very weak proportion the other aspects of metabolism and growth of ethyl-acetone bacteria. As to the actual manifestation of acetonic ability that depends principally on the presence of acetates. In consequence, there again we can register disagreement between conditions of medium indispensable for acquiring the determined physiological faculties and conditions necessary for their effective realization.

One finds in the literature, and notably in the works of the laboratory de Gabo (Gt. Britain) and of Mardechev (URSS) numerous analogous examples concerning conditions of formation and effective activity of different enzymes. We can also refer to our own observations which demonstrate the stadal character of the process of formation of conidia in mucedine, and also to research done at Meissel laboratory (URSS) which brought out the stadal character of production of riboflavin in *Eremothecium Ashbyii*. But lack of time does not permit us to go on at length. Let us hope that a study of specific needs of microorganisms in relation to the development of different structures and functions may be made on a very large scale. Such research will throw light on a number of problems and will facilitate the orientation of metabolism and development of microbes.